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CONTENTS

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PAGE

Editorial

Resistance to Invaders 103

General Articles

The Bovine Udder and its Agglutinins 105
W. R. Kerr, D.V.S.M., B.Sc., Ph.D., M.R.C.V.S.;
J. K. L. Pearson, Ph.D., B.Sc., M.R.C.V.S.; and
J. E. F. Rankin, B.Sc., M.R.C.V.S.

The Development of Resistance by Sheep to Infection with the Nematodes Nematodirus Filicollis and Nematodirus Battus 120
T. E. Gibson, M.V.Sc., M.R.C.V.S., Weybridge.

The Determination of the Blood and Plasma pH of the Ayrshire Cow and a Comparison with Similar Determinations in the Dog 123
E. W. Fisher, B.Sc., M.R.C.V.S., University of Glasgow Veterinary School.

A Device for Positioning Small Animals for Surgery 132
Bruce V. Jones, M.R.C.V.S., Veterinary Research Department, Allen and Hanburys Ltd., Ware, Herts.

Control of Haemorrhage in Canine Surgery 134
K. Cummings Sumner, M.R.C.V.S., Bristol.

The Use of the Zimmermann Reaction for Androgens and 17-Ketosteroids as a Possible Guide to Sexing Chicks 138
Albert Latif, Ph.D., Department of Animal Production, Faculty of Agriculture, Cairo University.

Reviews 141

Pallaske-Schmidel, Pathologisch-Histologische Technik
Evolution of the Veterinary Art

News 142

Mr. G. N. Henderson, B.Sc., M.R.C.V.S.

Notices 142

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EDITORIAL

RESISTANCE TO INVADERS

The most critical test of a clinician's skill rests in the examination of his patient after death. The report may bring a blush of pride or of shame, but no matter what may be the colour of his cheek his store of knowledge will have advanced.

Observation in the slaughter house and post mortem room will reveal evidence that animals do recover from diseases which, as a rule, are lethal in their effect or give rise to a state of chronic ill health. There are many instances in which there is no evidence that recovery has been induced by human aid or by specific chemo-therapeutic agents or by some biochemical product elaborated in a laboratory. In the light of present knowledge the idea that resolution can be explained by some spontaneous fortuitous change must be rejected, for such an explanation is the "refuge of the destitute" and as ludicrous as the ancient belief in the spontaneous origin of disease.

In many cases enquiry will show that there is no evidence of previous illness or abnormality and therefore as no treatment could have been applied it must be presumed that resolution was not incited by some external cause.

This leads one to the hypothesis that the beneficient results must have been induced by some factor normally present or generated within the compass of the victim's own metabolic forces. Since the time of Pasteur it has been recognised that with regard to those diseases which are of germ origin the body strains to resist an invader and substances known as antibodies are produced. The final result of the battle, be it good or bad for the patient, depends upon the ability of the latter to produce the requisite antibody and the potency thereof.

In simple terms, infective processes promote resistance and the final cure is due to the body's own response rather than to the action of any medicinal agent. Some agents stimulate natural resistance, others may hamper it. Resistance, active or passive,

depends in proportion upon the ability of the body to respond to stimulus, and good nursing can exercise a profound influence in this regard. Many able practitioners pay more attention to nursing than they do to drugs and with profit to the patient.

The attributes of resistance and the principles on which it is based are accepted by modern physicians in respect of those diseases which are of germ origin.

It is strange that the same phenomenon has not been accepted universally with regard to the troubles occasioned by parasites and malignant growths. There is evidence that both invaders can stimulate resistance. A visit to the dissecting room of any Veterinary College will demonstrate that many of the equine subjects in the dissecting room have been affected severely by parasitic infestations, particularly in the vascular system, and in spite of the fact that considerable structural damage has been occasioned, resolution and compensation have occurred so that the animals have survived in good condition for many years.

In recent years workers in the Glasgow Veterinary School have sought to produce a " vaccine " which would stimulate natural resistance to parasitic invasion, after the manner of that elicitated by vaccines in the case of bacterial infections.

They have worked on Parasitic Bronchitis in young cattle, which is caused by *Dictyocaulus Viviparous*. They have met with considerable success and the " vaccine " is prepared from living larvae which have been attenuated by irradiating them with X-rays. Their success ranks as one of the major medical discoveries and is a first step into a great field, for parasitic infestations rank high amongst the hazards to health and even life of man or animal.

There are other " parasites " which invade and destroy. Malignant growths, such as carcinoma, have much of the qualities of verminous parasites. There have been instances of resolution of carcinoma even when the case has been given up as hopeless. There must be many occasions of lesser degree when carcinoma has attacked and been repelled. The growths which develop to the stage of recognition must be those in which the power of natural resistance has failed. In the cases which do resolve, with or without surgical aid, it is probable that resolution is initiated by some " antibody " response. If antibody response can be encouraged in the case of verminous invasion, why not try to stimulate its production when the malignant " parasite " carcinoma invades?

THE BOVINE UDDER AND ITS AGGLUTININS

W. R. KERR, Ph.D., B.Sc., D.V.S.M., M.R.C.V.S.; J. K. L. PEARSON, Ph.D., B.Sc., M.R.C.V.S.; and J. E. F. RANKIN, B.Sc., M.R.C.V.S.

It is probable that the first scientific reference to this subject was made by Smith, Orcutt and Little in 1923. These workers published a paper " On the source of agglutinins in the Milk of Cows ". Their conclusions that the udder distinctly participated in the production of agglutinins when the gland is invaded by living or flooded by dead brucella organisms was at that time a contribution far in advance of the current conception of the local production of antibodies. Moreover it is doubtful if any serious thought has been given to it in the intervening years. They also recorded that a tissue reaction followed the infusion of the udder by these bacteria. Since 1923 reliable information on antibodies in the udder has been negligible, which is strange since so much has been published about colostrum (McGirr, 1947). Kerr and Robertson (1943, 1946, 1947 and 1953) and Kerr (1955) published a series of papers relating to the local production of antibodies in the uterus. Subsequently, it was suspected that there might be a parallel in the mechanism of immunity in the uterus and the udder and the authors of this paper, having been engaged for a number of years in the study of the milk agglutinins for *Br. abortus,* decided to extend their work and examine more fully the mode of production and possible value of the udder antibodies. This is a report of the various aspects of this work in the non-lactating and the lactating udder using several antigens.

The live and dead antigens used consisted of the organisms *Br. abortus, Salmonella pullorum,* coagulase-positive *Staphylococcus aureus* and *Trichomonas foetus.* A commercially produced staphylococcal toxoid was also used. It is the experience of the authors that almost all antigens are irritant to the lactating udder in varying degrees of severity as shown by the tissue reactions that follow their introduction. This reaction has been in all cases characterised by pain, swelling, tenseness and altered secretion, and sometimes by a high systemic temperature. The infused quarter recovers in two or three days but repeat infusions may eventually lead to permanent loss of the secreting function of the quarter.

The tissue reaction was, however, a major problem in that during the period of acute reaction serum globulins temporarily appear in the secretion and for the initial experiments an antigen which was non irritant was considered to be essential. *Trichomonas foetus* antigen was found to be satisfactory for this purpose; it is least irritant to the udder and is a non toxic antigen which induces a specific antibody.

The particular methods used are described in the various group histories. The serum and whey tests were orthodox, using standard antigens and were carried out as described in a previous paper (Kerr, Pearson & Rankin 1958).

Intramammary Experiments with Dead Trichomonad Antigen

The trichomonad antigen is relatively non toxic yet produces specific antibodies. It is soluble and diffuses readily. In the work with genital antibodies (Kerr and Robertson) it was shown that the dead trichomonad antigen was sufficiently soluble,

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or that the molecular size was sufficiently small, to pass the uterine barrier whereas larger and less soluble molecules particularly of precipitated antigens did not. One of the first requirements was to find whether these antigens would be retained in the udder or passed into the systemic circulation.

Five milking cows completely free from any trichomonad infection were used. As all the cows reacted in an identical manner it is not proposed to give the individual histories in detail but to give one as an example of the response of this group as a whole to an antigen of this type.

A preliminary explanation is made at this point because a normal serum titre line is shown in the graph. There is present in all bovine sera from animals 3 months of age and older an agglutinin to trichomonas which is non-specific, which maintains a constant level and which is an integral part of the serum. This phenomenon has been found with other protozoans and is now accepted as a non-specific agglutinin. Its presence means that any positive interpretation of the serum titre must be shown as a titre level that exceeds the normal agglutination line. The normal serum titre is not present in milk or whey from a normal healthy udder, but some titre is found in the secretion of the drying phase, and in colostrum, and in other circumstances where serum globulins appear in the milk. The trichomonad test is therefore a useful indicator as to when serum globulins occur in the milk and in this laboratory the test is used for this purpose.

Only one quarter was infused and the repeat infusions were made always in the same quarter. Infusions were made into the teat after milking, the antigen being suspended in 10 ml. of sterile physiological saline. The antigen was prepared by the freeze drying of centrifuged and washed culture of *Tr. foetus*.

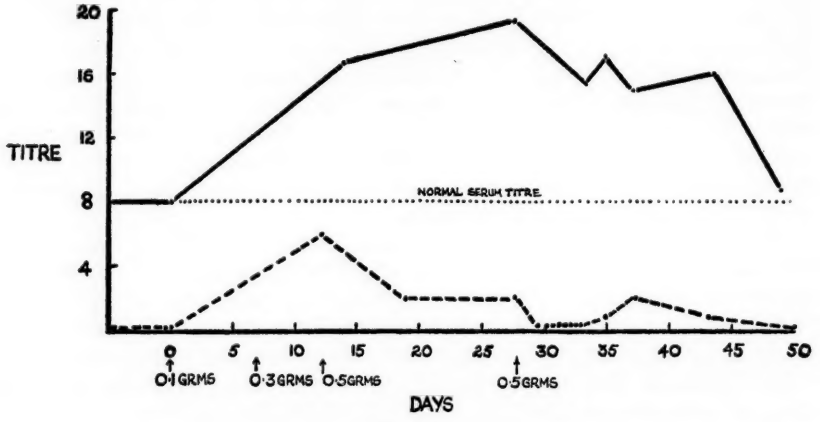

FIG 1.

The Response to Trichomonad Antigen.
The solid line denotes the serum titres and the broken line the whey titres.

The graph (Fig. 1) shows the response to trichomonad antigen. On the 12th day whey agglutinins reached an agglutination index of six, then steadily fell to an index of 2 which is a very low titre and may be regarded as little more than a trace.

This fall took place despite two further infusions. Following the 4th infusion the whey titre disappeared for 5 days and thereafter only a trace was found. The non-infused quarters followed a similar pattern and on one occasion the titre was even higher than in the infused quarter. However as the titres were so uniformly low the slight changes were not regarded as of significance.

The serum titre rose steadily to an index of 20 which is a high positive titre for serum and is exceeded only rarely in field cases of trichomoniasis.

When a non irritant soluble dead antigen is infused into the udder the antigen appears to pass through the udder barrier to the systemic system and induces a serum titre. That is the obvious interpretation from the graph but it is much more difficult to explain the presence of the low level agglutinins in the whey. One sample only, taken on the 13th day, showed appreciable agglutinins and thereafter only a trace was present. The important point in this connection is that the non-infused quarters carried similar titres. This would suggest that the titre in all quarters was of serum origin. The transitory presence of the agglutinins would also support this contention and is similar to the phenomenon which takes place in cows vaccinated subcutaneously with S.19 anti-abortion vaccine . There is no suggestion in this group of experiments with trichomonad antigen that antibodies had been induced locally.

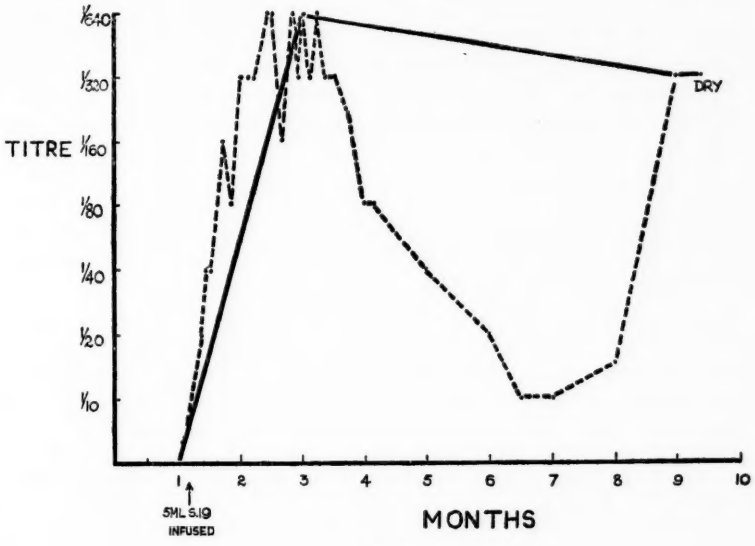

FIG 2.

The response to Live *Br. abortus.*
The solid line denotes the serum titres and the broken line the whey titres.

Intra-Mammary Experiments with Br. abortus Antigen

1. *Live Bacillary Antigen*

The infusion of live S.19 into the udder of 3 non-vaccinated cows, whose sera were negative in agglutination tests, was followed by a severe systemic reaction and a

rapid rise of temperature to 107°F. accompanied by all the signs associated with a high temperature. The local reaction of pain, swelling and altered secretion in the udder took place at four hours and reached maximum severity at six hours. Within three days the udder returned to normal shape and secretion. All three cows reacted similarly, therefore one graph (Fig. 2) is given of cow H22 illustrating the antibody response of this group.

The blood titres developed rapidly and reached a high level which persisted throughout the experiment. The whey titre at first equalled that of the blood. At 4 months however a steady fall in whey titre to 1-10 took place followed by a rapid rise to 1-320 as the cow became non-lactating some 2 months before her calving date. In the non lactating stage such agglutinins (*as S. pullorum* and *Tr. foetus*) were present and would suggest that in this cow the rise in agglutinins found in the drying-off stage was mostly due to a concentration in the udder of the serum globulins. This phenomenon is discussed further in a later stage of this paper.

Following the antigen infusion the agglutinins in the milk whey rapidly appeared in the non-infused quarters. This differed from our findings with dead brucella antigen, and the agglutination titres reached a level similar to that found in the infused quarters with only slight variations. The quarters with the greatest secretion showed a lower titre than the quarters with less secretion. The immediate appearance of agglutinins in the non-infused quarters would indicate that there was an invasion of organisms. An interesting finding in this experiment was that brucella organisms were not found in the milk microscopically, culturally or biologically after the 2nd day following the infusion. It is assumed that the infection at this stage becomes intracellular.

2. *Dead Antigen*

The antigen was prepared from a concentrated suspension of S.19 *Br. abortus* organism by washing with saline, precipitating with alcohol and subsequently drying the preparation with acetone and ether.

The most important feature of this group of experiments is that no transfer of antibodies took place from the infused to the non-infused quarters, therefore experiments with dead antigen are of considerable value in assessing the appearance of local and systemic antibodies. The alcohol precipitate of S.19 organisms is highly antigenic and 0.1 gramme subcutaneously will induce a high serum titre.

*Cow F.*14. Prior to the infusion of one quarter with the antigen all blood and milk titres were negative in agglutination tests at the lowest dilution of 1-5. The first infusion of 0.1 gramme suspended in 10 ml. of sterile saline was followed at six hours by the characteristic tissue reaction of pain, swelling and altered secretion. The udder returned to normal in 48 hours after the infusion. During the early period of the reaction milk samples were taken every two hours to examine the premise of Petersen (1957) that antibodies appeared in the udder secretion between four and 96 hours after exposure to antigen.

A serum titre of 1-5 was evident after five days and reached a partial agglutination in 1-80 at 11 days. Despite a second infusion of antigen the serum titre fell to 1-20 at 24 days. The whey titre was negative until the 17th day when a titre of 1/5

appeared for 24 hours only. Following the second infusion, (on day 21), a definite titre of 1-160 quickly appeared in the whey. The 3rd infusion completely neutralised that titre for 2 days; 24 hours following the neutralising the titre reached 1-1280. A gradual fall of titre then took place to 1-20 at 77 days when the 4th infusion (suspended in oil) was given and a further boost of the whey titre to 1-80 occurred. Some 160 samples were taken from the 3 non-infused control quarters and at no time was any titre detected except during a short period mentioned later when one quarter developed mastitis.

Two comments are made on this history. Firstly it was evident that following the first infusion the antigen had passed through into the systemic system resulting in a partial serum titre of 1-80 and although 0.4 gramme of antigen was subsequently infused, a very poor serum antibody response followed, which would suggest that a partial barrier, due to some change in the condition of the udder cells or that some interference with the antigen, had taken place. Secondly the whey titre shows

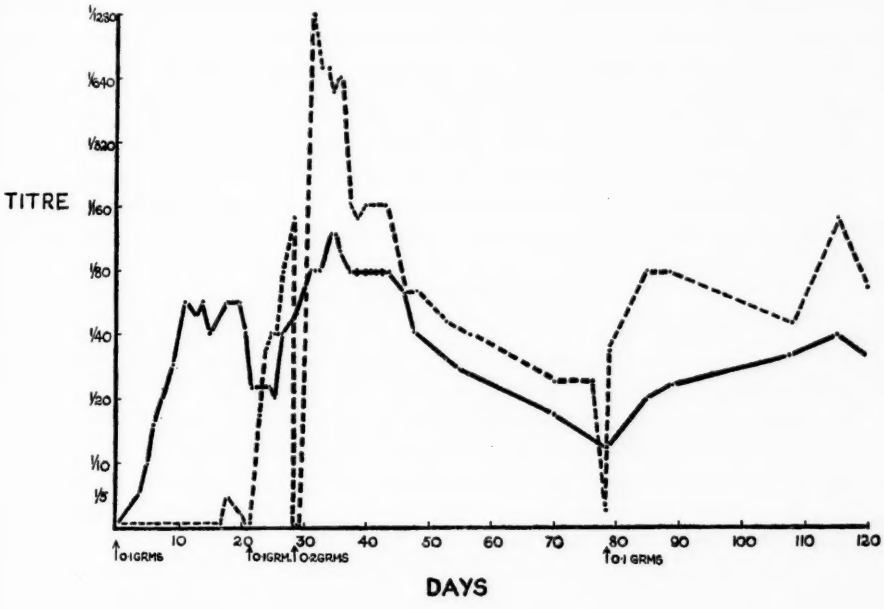

FIG 3.

The Response to Dead *Br. abortus* Antigen in a Lactating Cow.
The solid line denotes the serum titres and the broken line those of the L.F. quarter.
The control quarters (160) were all negative.

neutralisation following the infusions of the antigen particularly after the third when complete neutralisation took place for two days. As the cow was in full milk it is improbable that any free antigen existed in the quarter after a subsequent milking. It is more probable that the antigen was fixed on the cells and that the neutralisation

took place there. The immediate rise in whey titre following neutralisation would also suggest this.

For several reasons it is suggested that the antibodies in the udder had a local cellular origin. The non-infused control quarters failed to show a whey titre at any time so it is assumed that no infiltration of serum globulins had taken place, nor was there any question of transfer from the positive quarter to the control quarters. In the infused quarter other antibodies, e.g. *Tr. foetus* and *S. pullorum,* were not evident; these antibodies would have been detected if serum globulins had been present in that quarter. The fact that the brucella antibodies could be effectively neutralised is a strong argument in favour of the antibodies being of local origin. The relationship of the serum and whey titres is also of some significance.

The infusions caused damage to the infused quarter. After the quarter reaction subsided and the udder returned to normal the infused quarter secreted less milk; after the 4th infusion the secretion was reduced to 1 pint as compared with 4 pints in the other quarters.

One of the control quarters at the 9th day of the experiment developed an acute streptococcal mastitis which responded to treatment and recovered in two days. During these 2 days the secretion, which was markedly abnormal, contained in low concentration several antibodies including those to *S. pullorum, Tr. foetus* and *Br. abortus.* All these antibodies were, of course, present in the cow's serum and the obvious inference is that serum globulins escaped into the affected quarter thus giving rise to the agglutination titres exhibited by the secretion. Immediately the quarter recovered the serum antibodies disappeared from it and the whey remained negative for the rest of the experimental period.

Staphylococcal Toxoid Group

Four cows were infused as follows:—

1. 2 ml. of commercially produced toxoid undiluted into 1 quarter. The infusions of the undiluted toxoid were followed by severe clinical reactions. This reaction was so definite that it was decided to dilute the toxoid with saline to 1-25 in the two subsequent weekly infusions, an amount which is almost impracticable in application.

2. 2 ml. of toxoid prepared in this laboratory was diluted 1-10 and infused into 1 quarter followed by three repeat infusions made at weekly intervals. This was accompanied by tenseness, pain and an altered secretion.

3. 2 ml. commercial toxoid diluted 1-20 were infused into 1 quarter followed by three repeat infusions made at weekly intervals. There was a severe reaction, but in this case a more transient one followed each infusion.

4. 2 ml. of locally-prepared toxoid diluted 1-10 were infused into all 4 quarters, with a repeat infusion of 1 ml. into each quarter at 7 days. A transient and fairly severe reaction followed and the cow went prematurely dry — a not uncommon sequel to infusing foreign substances into the udder.

No antitoxin was detected in the milk following the infusion of toxoid into the udder of lactating cows. However when these particular cows entered the dry phase antitoxins were found to be present in the secretion and it is assumed for certain

reasons that these antitoxins were of serum globulin origin. Firstly, because the sera of these cows showed a partial 1-16 titre prior to the experiment and as is discussed elsewhere in this paper the serum antibodies are found to be present in the udder secretion when the cow is in the dry phase. Secondly, the antitoxins appeared equally in all quarters which is the result found when serum globulins permeate into the udder. This result definitely differs from that which occurs when antibodies are produced locally in one quarter. Finally, after the colostral phase the serum-globulin antitoxins disappear; this would not be the case if only locally produced antitoxins were present. It is the opinion of the authors that detectable amounts of antitoxin were not produced in the lactating udder in these experiments.

Experiments with Salmonella pullorum Antigen

Salmonella antigens were included for the purpose of examining Petersen's (1957) premise that the udder of the milking cow when exposed to small numbers of live *S. pullorum* showed the early presence of antibodies. He found antibodies present at 4 hours in the secretion from the fore quarters and at six hours in the hind quarters. At 96 hours he found secretions from all quarters to be strongly positive in agglutination tests.

A. Live Salin pullorum Antigen in the Lactating Cow

The cow had the usual serum titre of 1-16 and milk titre negative for *S. pullorum* prior to the experiment. The first infusion was of a live avirulent culture of *S. pullorum* (1 million organisms in 10 ml. saline) into one quarter. Practically no clinical reaction or serological response followed the infusion. The serum titre rose one dilution to 1-32 but the daily milk-whey samples were definitely negative in dilutions of 1-4 for a period of sixteen days when 750 million organisms of a freshly isolated field strain were infused into the same quarter. This was followed by a sharp clinical reaction, the udder became enlarged and secreted a yellow coloured clotted milk. The temperature rose to 105.6°. The reaction subsided in 24 hours during which time samples were taken at 2 hourly intervals. Thereafter samples were taken daily for 3 weeks. The blood titre remained constant at 1.32. The whey agglutination tests were completely negative in samples from all four quarters; that is, at no time in this experiment was agglutinin demonstrated in the milk.

The live organisms apparently did not remain in the udder as agglutinins were not formed nor were positive cultures obtained from milk after 48 hours.

B. Dead Salin pullorum Antigen in the Lactating Cow

In this experiment alcohol precipitated *S. pullorum* was made in a similar manner to the *Br. abortus* antigen. The antigen, in 10 ml. of saline, was infused into one quarter of the udder, the primary dose being fixed at 0.1 g.

The reactions that followed the infusion of the antigen were similar to those that followed the *Br. abortus* antigen; the systemic reaction was slightly less severe.

The initial serum titre was 1-16, a normal titre for the bovine, the whey was negative in 1-4 dilution. The second infusion of 0.1 g. antigen was made 7 days later when it was shown that no whey titre had developed nor had any increase in serum titre taken place. On the 12th day the serum titre rose to 1-64, but the whey

titre of the infused quarter remained negative. On the 15th day a partial whey titre of 1-4 was found. A third infusion of 0.1 gm. was then made at the 15th day. From that date there was a steady increase in serum and whey titres to the 26th day when both reached 1-1024. A fourth infusion of 0.1 g. was made on the 27th day. This was immediately followed by a partial neutralisation or reduction of the whey titre. The titre during the neutralisation fell from 1.1024 to 1-64 and 24 hours later returned to its previous level. The serum titre remained constant. In the absence of further infusions both serum and milk titres fell steadily for 2 months when serum exhibited a titre of 1-64 and milk-whey 1-16. The antibody responses are shown graphically in fig. 4.

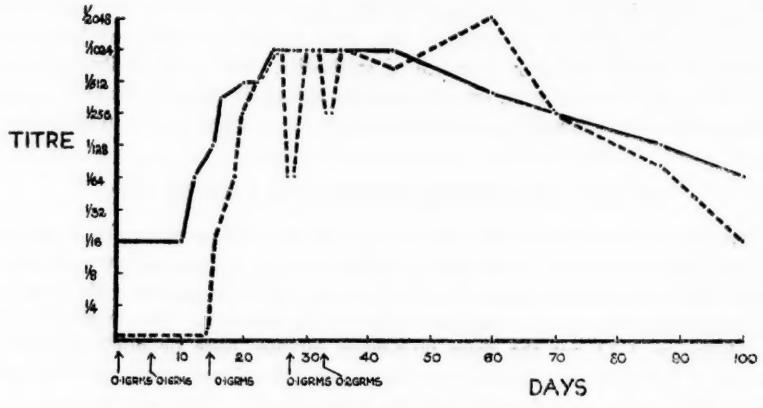

FIG 4.

The control samples (90) were all negative.

The Response to Dead *Salm. pullorum* Antigen in a Lactating Cow.

The solid line denotes the serum titres and the broken line the whey titres.

The neutralising phenomenon of the udder antibodies was less spectacular than that which occurred with the brucella antigen. This could result from a variety of reasons, principally the quantitative relation of antigen to the milk content of the quarter and the fact that it is an antigen-antibody junction. Also the molecular size may be concerned.

The 90 control milk samples from non-infused quarters were completely negative throughout the experiment. In the experiment with the same antigen in dry cows antibodies did appear in the control quarters due to the presence of serum globulins in the secretion. This is discussed later in the dry cow experiments.

The development of udder antibodies to *S. pullorum* in the milking cow followed a pattern similar to that which had followed the brucella infusions.

C. Dead Salin pullorum Antigen in the Non-lactating Cow

Alcohol precipitated *S. pullorum* antigen was infused into one quarter of a cow 7 months pregnant and non-lactating, with a serum titre of partial agglutination in 1:16 and udder secretion practically negative prior to infusion.

The udder reaction to the 0.1 g. of antigen was slight, without tenseness or pain but with some enlargement. The secretion became yellow coloured and contained a large quantity of sediment. The reaction disappeared in 24 hours. No agglutinins were present in the early two-hourly samples of the udder secretion nor in the subsequent daily samples. At the 9th day the serum titre rose to 1-64 and the secretions of all quarters to a titre of 1-4 (fig. 5).

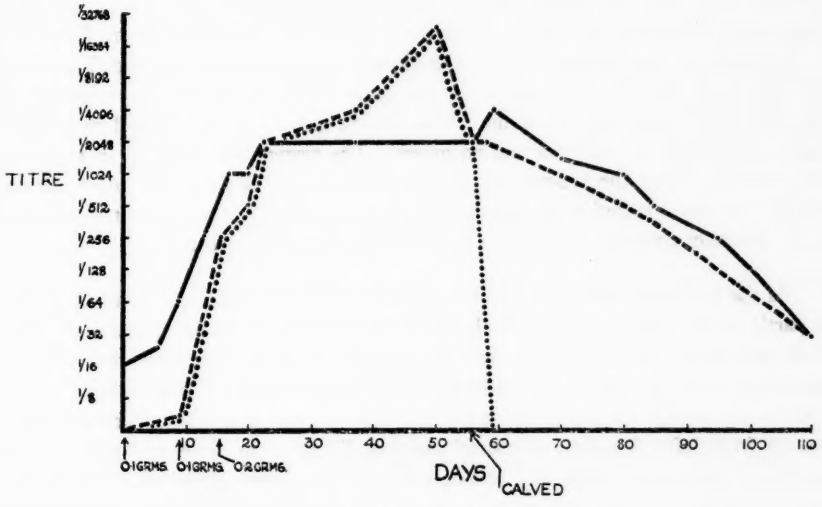

FIG 5.

The Response to Dead *Salm. pullorum* Antigen in a Non-lactating Cow. The solid line denotes the serum titres, the broken line those of the infused quarter, and the dotted line those of the control quarters.

The antibody content of the blood and of milk from the control quarters and the infused quarter continued to rise steadily in parallel until the 23rd day. Thereafter as parturition approached the secretion titres continued further to rise to a partial agglutination in 1-32,000, all quarters being approximately equal. The serum titre did not rise at this period.

On the 56th day the cow calved and twelve hours later the titre of the colostral whey from all four quarters was 1-2048.

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On the second day following calving the agglutination titre of the secretion from one control quarter became negative and the titres of the two other quarters fell to 1-256. On the third day after calving the titres of the whey from all control quarters were negative whilst the infused quarter remained positive with a titre of 1-2048. Although the titre fell steadily it still remained high and at six weeks a titre of 1-32 was recorded.

During the post-colostral period there was no evidence of serum globulin agglutinins in the whey of the infused quarter samples. Only the specific *S. pullorum* agglutinins were found, suggesting that these were of local origin.

The most important observation of this experiment is that the secretion from the control quarters, which had developed a high titre in parallel with the infused quarter secretion and with the serum during late pregnancy, ceased to show antibodies immediately colostrum production terminated. This is a good example of the effect of the barrier in the milking udder. Examination of whey from the control quarters and also from the infused quarter at this stage failed to reveal the presence of agglutinins associated with the serum. The infused quarter sample showed its specific agglutinins against *S. pullorum* and no others. The tremendous build-up in antibody concentration during the pre-colostral period, followed by the rapid fall which occurs with the increased secretion of colostrum is shown in fig. 5. The volumetric dilution of the antibody content is considered to be an important factor here.

In the dry phase there was no real evidence that the antigen was retained totally or partly in the udder. From a study of the serum titres it would appear that most of it had passed through to the systemic system but it is also clear that specific agglutinins were present in the infused quarter when sampled in the milking stage, whereas the control quarters contained none. It was shown in earlier experiments that passage from infused quarter to control quarters did not take place, and that control quarters if they show agglutinins receive them via the serum globulins. During the non-lactating period there appears to be a fairly free movement of antigen and antibody from udder to circulatory system and vice versa.

One other important feature in these experiments is that antigens produce considerably less tissue reaction in the non-lactating udder than in the milking udder.

Immunisation of the udder may therefore be practicable only during the dry phase unless a method can be introduced whereby the antigen is rendered much less irritant to the udder tissue but not less antigenic.

Absorption Experiments on Secretions from Udders of Non-lactating Cows

Colostrum and its concentration of antibodies is fully appreciated in the literature but not so much evidence is available with regard to precolostrum. During the work

of the brucellosis survey (Kerr, Pearson, Rankin, 1958) it was found that the secretions from non-lactating udders at varying stages of preparedness for parturition contained antibodies for *Br. abortus, S. pullorum, Tr. foetus* and *Staph. aureus* provided that the blood at the same time also contained those antibodies. The antibody picture of the serum is therefore important. A cow not infected with brucella and not vaccinated will not exhibit brucella agglutinins in the dry udder secretion. All dry cows tested had some salmonella agglutinins probably due to the fact that fraction XII is a shared antigen with other organisms. It is not unexpected therefore that cows may carry a low serum titre to *S. pullorum*. With regard to *Tr. foetus* antibodies the presence of the natural agglutinin in serum has already been discussed.

Simple absorption experiments were carried out in the dry udder secretions with specific antigens to determine whether or not these specific antibodies could be removed, leaving the other agglutinins more or less in their original concentration.

The absorption was carried out by mixing the secretion with a concentrated suspension of washed bacterial cells, incubating at 37°C. for 2-4 hours, centrifuging and filtering if necessary to clear the supernatent fluid, then subjecting this fluid to agglutination tests.

From these experiments it is concluded that the specific agglutinins to *Br. abortus* and *S. pullorum* in the precolostrum and colostrum can be absorbed by their specific antigens, and that such absorption leaves the amounts of other antibodies almost unaltered.

Some examples are shown in the Table.

Cow H. 12	Titre before absorption with *Br. abortus* cells	After absorption with *Br. abortus* cells
Br. abortus	1:40	Nil
S. pullorum	1:16	Partial 1:16
Tr. foetus	Index 4	Index 4
S. aureus beta antitoxin	32 units	32 units

Cow H. 12		Titre before absorption with *Salin. pullorum* cells	After absorption with *Salin. pullorum* cells
Br. abortus) quarters	RF	1:40	1-20
antigen)	RH	1:20	1-20
	LF	1:40	1-20
	LH	1:20	1-20
S. pullorum quarters	RF	1:16	Nil
	RH	1:16	»
	LF	1:16	»
	LH	1:16	»

Cow B. 1 (Bulk udder secretion) (4-6 weeks before calving)	Titre before absorption with Brucella antigen	Titre after Brucella absorption Nil (i.e. less than 1/10)
Br. abortus antigen	1/40	Nil
Salmonella pullorum antigen	Nil	Agg1. index 7-8
Tr. foetus antigen	Aggl. index 8	2 units
Staph. aureus beta antitoxin	2 units	

Cow 173	(4 *weeks before calving*) Titre before absorption with Brucella antigen	Titre after absorption
L.F. quarter		
Br. abortus antigen	1/20 (partial 1/40)	Nil
Staph. antitoxin	16 units	8 units
Salin. pullorum antigen	Nil	Nil
R.F. quarter		
Br. abortus antigen	1/20	Nil
Staph. antitoxin	16 units	16 units
Salin. pullorum antigen	Nil	Nil

(Samples were available from 2 front quarters only)

Cow F. 4 (Colostrum taken) (at parturition)		Titre before absorption with Brucella antigen	Titre after absorption
Br. abortus antigen	L.F.	1/20	Nil
	L.H.	1/20	Nil
	R.F.	1/20	Nil
	R.H.	1/20	Nil
Salin. pullorum antigen	L.F.	Rapid plate test—Pos.	Rapid plate test—Pos.
	L.H.	do. —Pos.	do. —Pos.
	R.F.	do. —Pos.	do. —Pos.
	R.H.	do. —Pos.	do. —Pos.
Tr. foetus antigen	L.F.	Aggl. Index 9	Aggl. Index 9
	L.H.	„ 8	„ 8
	R.F.	„ 8	„ 9
	R.H.	„ 9	„ 9
Staph. antitoxins	L.F.	Nil	Nil
	L.H.	„	„
	R.F.	„	„
	R.H.	„	„

Cow F. 6 Pre-colostrum, taken 24 hrs. before calving		Titre before absorption with *Br. abortus* antigen	Titre after absorption with Brucella cells
Br. abortus antigen	L.F.	1/10	Nil
	L.H.	1/20	Nil
	R.F.	1/10	Nil
	R.H.	1/40	Partial 1/10
Salin. pullorum antigen	L.F.	1/16	1/16
	L.H.	1/32	1/16
	R.F.	1/16	1/16
	R.H.	1/32	Partial 1/32
Tr. foetus antigen	L.F.	Index 12	Index 11
	L.H.	„ 14	„ 13
	R.F.	„ 11	„ 10
	R.H.	„ 15	„ 15
Staph. antitoxins		Each quarter nil	Each quarter nil

Discussion

One of the obvious features of this study is that the mammary gland was found to be an extremely sensitive organ which reacts somewhat violently to the infusion of its quarters with antigenic substances. These reactions follow a common pattern of pain, swelling, tenseness, heat and altered secretion and in some cases a high temperature. Several such reactions following one another lead to the quarter becoming prematurely dry with probable permanent loss of the quarter as the induration is fibrotic and extensive repair is unlikely. Certain reservations must be made, however, as some less toxic antigens, for example trichomonas, were found not to excite such a severe local reaction. Another point is that attenuated live bacilli may be less irritant than their virulent counterpart.

It is important to note however that a number of infusions are required to attain the necessary immunity level and the subsequent damage caused by these multiple infusions makes this approach impractible unless future work could evolve methods, chemical or otherwise, of buffering antigens so that they may induce an immunity without causing excessive tissue damage.

Whilst these experiments were carried out in the first place to examine the possibility of local production of antibodies and to see if these antibodies could be applied in the control of mastitis infections, our other particular interest in the work was that of the appearance of brucella agglutinins in the milk and their significance in the interpretation of the diagnostic tests for brucellosis. There are several occasions when brucella agglutinins of serum origin appear in the milk and as such constitute false positives. The reverse of this is that milk from *Br. abortus* blood positive cows may be culturally and biologically positive and yet be agglutinin negative. This observation by Roepke (1957) has been confirmed by the authors. The fact that the height of the whey titres and the presence of organisms may not be directly related is also of interest.

XUM

The literature is extremely scarce on the subject of udder antibodies and the work of Petersen (1957) in which he states that the udder of the cow is a veritable factory of antibodies in the dry and milking phases is of interest. Our work indicates that the udder can be stimulated to produce antibodies but that the production of these locally made agglutinins is a slow process and it requires multiple infusions, especially with a dead antigen. The antibodies so produced may be neutralised by their specific antigens.

It is shown also that antibodies pass freely from the serum to the secretion of a non-lactating udder and that these may be absorbed by their specific antigens. There is evidence to indicate that the antibodies do not pass directly from one quarter to another. The serum titre appears to be unrelated to the whey titre in the milking cow.

An observation is made that, as in the uterus, a barrier-like effect exists in the udder and serum agglutinins do not appear in the milk except under certain recognised circumstances. It was expected that precipitated antigens would probably be held by the udder cells, but the impression has been gained now that the first antigen infusion is followed by free absorption into the systemic circulation and thereafter there is built up a resistance to the passage of the antigen. At least the systemic responses seem to be less to subsequent infusions. The neutralisation which occurs presents more positive evidence of this phenomenon. For a period of 24 hours to 48 hours the infused antigen, despite frequent milkings, neutralised or blocked the agglutinins secreted during that period. This was followed by a rapid step up of the agglutination titre. This evidence is also supported by the fact that the agglutinins in the whey can be absorbed out in vitro by their specific antigen. Another interesting point is that the agglutinins only enter the non-infused quarters at the drying-off phase and not by passages from quarter to quarter. The titres in the control quarters all approximate to each other and are closely related to that of the serum. When the colostral phase terminates this serum globulin agglutinin content disappears from the control non-infused quarters (fig 5). Moreover during the long period that the whey titre is present in the infused quarter when in full milk it has been shown that titres of *S. pullorum* and *Tr. foetus* are not present in any quarter. This indicates that the serum is not the source of the antibody. It is suggested that this agglutinin is the product of the udder cells, probably plasmocytic. It is also accepted that at the dry phase and the colostrum stage there are two separate groups of antibodies present in the infused quarter — the locally produced and those of serum origin — and that after colostrum secretion terminates the serum titre is excluded leaving the locally produced antibody. The use of *Tr. foetus* and *S. pullorum* antigens is a useful method of testing for the presence of serum globulins.

At the moment the authors are not optimistic about the probability of evolving an antigen that is actively antigenic but non-irritant.

The dry udder is apparently the best stage however to introduce an antigen, as at this stage it does least damage and produces both systemic and local antibodies. It is not possible however to say that irreparable damage may not be done as it is essential

to infuse three times to induce a high titre. Some future work may determine the value of this immunity from a protection point of view.

The lactating udder is much more difficult to immunise, the tissue reaction is more severe and the antibodies more difficult to induce than is the case with the non-lactating gland.

Summary

Experiments have been carried out in which bacterial and protozoan antigens were infused into lactating and non-lactating udders of cows. Evidence is advanced to demonstrate that a series of infusions could stimulate the production of specific antibodies within the gland.

The disturbance to the animal caused by the infusions is described and there is discussion upon the practicability of using this procedure as a method of immunising against udder infections.

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XUM

THE DEVELOPMENT OF RESISTANCE BY SHEEP TO INFECTION WITH THE NEMATODES NEMATODIRUS FILICOLLIS AND NEMATODIRUS BATTUS

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Although *Nematodirus* spp. are of frequent occurrence in sheep in this country nematodiriasis occurs only in lambs under ten weeks of age. Lambs dying of the disease carry heavy burdens of worms of *Nematodirus* spp. but older sheep when examined *post-mortem* are rarely found to be carrying more than a few hundred mature worms. These observations suggest that a resistance to infection with *Nematodirus* spp. develops in lambs at about the age of two months. The experiments described in this paper were undertaken to decide whether age resistance to *Nematodirus* spp. develops in lambs and whether acquired resistance is an important factor in the resistance displayed by mature animals.

Materials and Methods

Seven sheep were used to test the development of age resistance, their ages at the beginning of the experiment being No. 1 eight weeks, No. 2 twenty-eight weeks, No. 3 forty-two weeks, No. 4 sixty-nine weeks and Nos. 5-7 eighty-nine weeks respectively. All these sheep had been kept from birth under strict conditions of isolation and, at the beginning of the experiment, were free from infestation with gastro-intestinal nematodes. Each sheep was given 500,000 infective larvae of *Nematodirus* spp. of which twelve per cent. were *N. filicollis* and eighty-eight per cent. *N. battus*. The first five lambs were killed for post-mortem examination four weeks after infection, but No. 6 was killed eight weeks and No. 7 twelve weeks after infection. At post-mortem examination the abomasum, the small intestine and the large intestine were removed from each sheep and the number of worms present in each viscus determined by the usual dilution method. Faecal egg counts were carried out daily during the observation period using the modified Clayton Lane flotation method.

Two other sheep, Nos. 8 and 9, were used to test the effect of previous infestation on the development of resistance. Sheep No. 8 was infected with 100,000 infective larvae of *Nematodirus* spp. when eight weeks old and its resistance was challenged by the administration of 500,000 infective larvae at 106 weeks of age. Sheep No. 9 received a dose of 50,000 infective larvae of *Nematodirus* spp. at six weeks of age and a test dose of 500,000 infective larvae was administered to challenge its resistance at sixty-four weeks of age. Both these sheep were slaughtered four weeks after the administration of the challenging infestation and the alimentary canal examined for the presence of nematodes by the usual dilution method. Faecal examinations were carried out again during the experimental period, by the modified Clayton Lane method.

XUM

Results

The results have been summarised in Table 1 from which it will be seen that both species of *Nematodirus* behave in a similar fashion. Details of the faecal egg counts have not been included as in all animals except sheep No. 7 eggs were passed on only a few occasions in very small numbers. Sheep No. 7 began passing eggs three weeks after infection and a week later the count had reached 200 eggs per gram of faeces. Two weeks later, however, the egg count had fallen to less than 50 eggs per gram and remained very low during the rest of the period of observation.

Discussion

From the table it is seen that the eight weeks' old lamb had no resistance to infestation with *N. battus* or *N. filicollis*. The worms established themselves and developed to maturity at the normal rate, egg laying by the females commencing some two weeks after infection. The lamb displayed no resistance to the effects of the infestation for it died $2\frac{1}{2}$ weeks after infection.

The older sheep behaved differently. The heavy worm burden of sheep 2, 4 and 5 indicates feeble resistance to the establishment of infestation in these animals but the absence of clinical response demonstrates a strong resistance to the effects of infestation. Sheep No. 6, killed eight weeks after infestation had a lower worm burden than No. 5, killed at four weeks and sheep No. 7, killed at twelve weeks had an even lower worm burden than No. 6. It is noteworthy that in these two sheep about two thirds of the worms were mature. From these observations it is reasonable to deduce that mature sheep possess little resistance to the establishment of infestations of *N. battus* and *N. filicollis* but that they do possess the power of inhibiting the development of the worms and subsequently of eliminating the greater part of the worm burden which initially becomes established. Sheep No. 3 which had a significantly lower worm burden than Nos. 2, 4 and 5 does not fit into this general picture but the fact that this sheep was carrying a small number of mature worms indicates that the same mechanisms were operating but that they were proceeding at a much faster rate. Differences of this kind between animals of a small group are of common occurence in helminthological investigations and can only be eliminated by the use of larger groups than is normally practicable.

Tetley (1935) was able to establish a light infestation in a ten months old sheep, which had been kept, till then, free from infestation, by grazing it on an infected pasture and he concluded that age resistance to *Nematodirus* spp. did not develop in sheep. Although the present observations also demonstrated the possibility of establishing infestations in mature animals they do not support Tetley's conclusion but indicate that a form of age resistance does occur in sheep. Tetley also concluded, from other observations, that infection with *Nematodirus* spp. produced strong resistance to superinfection, but this is not supported by the present experiments for it proved possible to infect sheep Nos. 8 and 9, which had experienced previous infestation to a degree similar to those which had not.

The present experiments indicate that a form of age resistance to *N. battus* and *N. filicollis* occurs in sheep and clearly it is of advantage to take steps to prevent the acquisition of heavy infestations by young lambs if clinical disease is to be avoided. It

XUM

TABLE 1. A summary of the results of the experiment.

Sheep No.	Age in weeks at time of infection	Weeks after infection when killed	Worms recovered at post-mortem examination						Duration of patency of the infection	Clinical effect on lamb
			Nematodirus filicollis		*Nematodirus battus*		Total *Nematodirus* spp.			
			Immature	Mature	Immature	Mature	Immature	Mature		
1	8	2½*	2,800	13,600	600	39,100	3,400	52,700	2 weeks after infection until death	Lamb died
2	28	4	6,200	0	56,100	0	62,300	0	Infection did not become patent	No obvious clinical effect
3	42	4	3,200	200	300	0	3,500	200	„	„
4	69	4	7,300	0	114,500	0	121,800	0	„	„
5	89	4	5,100	0	24,900	0	30,000	0	„	„
6	89	8	2,600	5,000	0	0	2,600	5,000	7 weeks after infection until death	„
7	89	12	1,400	2,200	0	300	1,400	2,500	3 weeks after infection until death	„
8	106**	4	0	0	68,400	0	68,400	0	Infection did not become patent	„
9	64***	4	4,900	2,400	16,000	0	20,900	2,400	3 weeks after infection until death	„

* Died.

** Had previously been infected with 100,000 larvae when 8 weeks old.

*** Had previously been infected with 50,000 larvae when 6 weeks old.

is also evident that this procedure does not leave them highly susceptible if exposed to infestation later in life.

Summary

Experiments carried out on worm-free sheep, whose ages ranged from 8-89 weeks showed that sheep over six months of age were resistant to the effects of infestation with *Nematodirus* spp. Although larvae were able to establish themselves in mature sheep their development was inhibited and eventually fewer worms reached maturity than in the susceptible eight weeks' old lamb. In the second part of the experiment previous infestation appeared to convey no significant immunity. The significance of these findings on the control and epidemiology of nematodiriasis is discussed.

Acknowledgment

Thanks are due to Mr. G. Everett and Mr. R. Parker for technical assistance in the course of this work.

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THE DETERMINATION OF THE BLOOD AND PLASMA pH OF THE AYRSHIRE COW AND A COMPARISON WITH SIMILAR DETERMINATIONS IN THE DOG

by

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At the present time the majority of the measurements of the pH of body fluids are made electronically using specialised electronic equipment and specialised electrode systems. However, technical difficulties arise when using most pH meters at temperatures of 38°C. unless they and the electrode systems are kept and used in a room at this temperature.

Various colorimetric methods have been described (Hastings and Sendroy, 1924; Shock and Hastings, 1934; Rutledge, 1948; Nadeau, 1953). Van Slyke *et al.* (1949) described a method using phenol-red as indicator with a Coleman Junior Spectro-

XUM

photometer for reading the optical densities. They claim an accuracy of ±.02 pH units which is equal or better than that given by many commercial pH meters.

Rosenthal (1948) showed that unless separation of cells and plasma was carried out at 38°C., the value obtained for plasma pH by measurement at 38°C. of a sample separated at a lower temperature would be higher than the actual pH in circulating blood due to the difference in temperature coefficients of whole blood and plasma.

Many pH determinations have been carried out on bovine blood and plasma usually obtained without stasis from the jugular vein (Bianca, 1955; Krapf, 1940; McSherry and Grinyer, 1954; Dale and Brody, 1954; Craige *et al.*, 1949). Both colorimetric and potentiometric methods have been used. There are, however, few records of pH determinations in Ayrshire cattle (Bianca, 1955) and no record of a number of pH determinations on the blood and plasma of Ayrshire cattle.

When similar determinations are made in the human subject, the importance of making pH determinations on arterial or arterialised blood or plasma is stressed (Davenport, 1950). Since a method of obtaining arterial blood from cattle for routine purposes has been found (Fisher, 1956), it was decided to determine the true plasma pH at 38°C. of arterial blood from Ayrshire cows and in doing so to determine temperature coefficients for blood and plasma.

It was decided also to determine the pH of ox plasma from venous blood samples obtained from Ayrshire cows and, for purposes of comparison, to find the pH of the arterial plasma of a non-ruminant animal, the dog.

Methods

(i) Arterial samples from the ox were taken by direct puncture of the brachial artery.

Venous samples were taken into an oiled syringe without stasis either from the jugular vein or from a catheter in the right atrium. The samples were collected under liquid paraffin into heparinised centrifuge tubes. The tubes were sealed immediately with a rubber bung leaving the minimum amount of oil between the blood and the bung. The tubes were either centrifuged at once or placed in ice for centrifugation a few hours later.

Arterial samples were taken from the femoral artery of dogs. For each pH determination duplicate samples were taken to avoid sampling errors and duplicate estimations were carried out on each sample. For the determination of temperature coefficients four samples were taken from each animal and these were centrifuged immediately (two at 0°C. and two at 25°C.)

(ii) Blood and plasma pH.

The method of Van Slyke (1949) has been used for the determination of plasma pH adapted to an EEL colorimeter. This is an easily available piece of equipment with cheap colorimeter cells.

Reagents

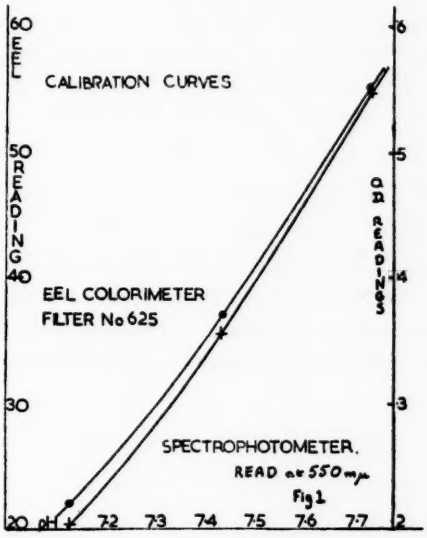
All the reagents are made as directed by Van Slyke. All measurements are made direct into the 8 ml. size colorimeter tube which in fact can hold about 11 ml.

Buffer mixtures and samples are covered with a thin layer of neutralised mineral oil and the tubes are stoppered.

As reported by Van Slyke (1949) the calibration curve read at 550mμ is not quite linear. He drew the nearest straight line to the three points.

Comparison of curves produced on the EEL colorimeter using a number 625 Ilford filter, and a spectrophotometer reading optical density at 550mμ shows that a similar curve is produced with both instruments together with a similar scale width (fig. 1). If the calibration curve is read at two specific wavelengths (420mμ and 565mμ) on the spectrophotometer and ratios of the optical densities at these wave-

FIG. 1



lengths are plotted on semi-logarithmic paper, a straight line is produced (fig. 2) (Ruthedge, 1948). It was discovered that a straight line of slightly greater scale width could be obtained with the EEL colorimeter using a simple glass filter O.G.R.I. (fig. 3). To ensure that the operation could be repeated it was found necessary to be certain that the colorimeter readings were made at a constant temperature. The assumption that room temperature was constant at 20°C. was found to be erroneous and a source of error. By holding the buffer mixtures and samples at 21°C. or 39°C., depending on temperature at the time of reading, it was possible to eliminate thermal effects and to read at 20°C. and 38°C. Once in the EEL colorimeter the temperature drop took 20 seconds allowing ample time to take a reading.

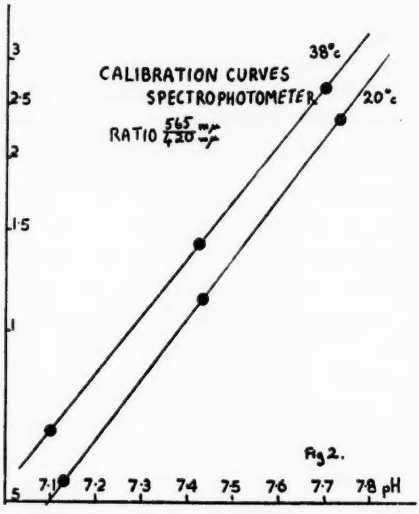
Results so far obtained give good agreement between duplicates. Consecutive determinations were then carried out on 10 samples. The results obtained are shown in table 1.

XUM

(iii) Determination of temperature coefficients.

Rosenthal (1948) showed that there was a difference in temperature coefficients between plasma and whole blood and that there were species variations in the temperature coefficients of plasma.

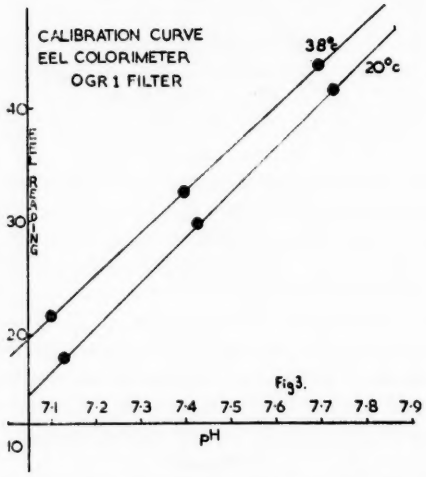
FIG. 2



He also showed that these coefficients were linear over the usual pH range.

(a) Temperature coefficient of plasma. This was determined by reading each plasma sample at two different temperatures in the colorimeter and so the difference

FIG. 3



in pH values for the difference in temperature could be determined. By division the temperature coefficient may be obtained.

TABLE 1

Examination of error of consecutive determinations on the same sample. Results Uncorrected.

Sample	a	b	c	d	e	f
1	7.695	7.705	7.695	7.695	7.695	7.695
2	7.670	7.670	7.655	7.655	7.655	7.655
3	7.680	7.675	7.680	7.675	7.680	7.680
4	7.490	7.485	7.485	7.485	7.485	7.485
5	7.615	7.625	7.625	7.615	7.630	7.630
6	7.410	7.430	7.430	7.430	7.425	7.425
7	7.780	7.770	7.755	7.770	7.770	
8	7.755	7.755	7.780	7.780	7.770	
9	7.740	7.725	7.740	7.740		
10	7.770	7.780	7.780	7.780		

(b) Temperature coefficient of whole blood. With no facilities available for the determination of the pH of whole blood or for centrifugation at 38°C., it has been found possible to determine the pH of whole blood indirectly and to determine the temperature coefficient of whole blood.

TABLE 2

Temperature Coefficient of Bovine Plasma (33 *samples*)

Sample	pH 20° C.	pH 38° C.	Difference
1	7.605	7.465	.140
2	7.630	7.485	.145
3	7.665	7.510	.155
4	7.680	7.520	.160
5	7.720	7.570	.150
6	7.670	7.455	.115
7	7.695	7.490	.205
8	7.740	7.555	.185
9	7.745	7.560	.185
10	7.635	7.495	.140
11	7.645	7.510	.135
12	7.705	7.545	.160
13	7.660	7.535	.125
14	7.615	7.465	.150
15	7.645	7.490	.155
16	7.680	7.530	.150
17	7.650	7.495	.155
18	7.640	7.470	.170
19	7.650	7.495	.155
20	7.705	7.555	.150
21	7.655	7.570	.145
22	7.690	7.530	.160
23	7.635	7.490	.145
24	7.775	7.595	.180
25	7.740	7.580	.160
26	7.640	7.450	.190
27	7.660	7.575	.145
28	7.660	7.495	.165
29	7.655	7.525	.130
30	7.570	7.420	.150
31	7.450	7.290	.160
32	7.600	7.430	.170
33	7.650	7.405	.145

Mean difference .155 for 18° C.

Therefore coefficient = .009 pH units per degree centigrade.

The pH of whole blood and plasma is the same at the temperature of centrifugation since at this temperature equilibrium exists between the cells suspended and the plasma. If two identical samples be taken and centrifuged at two different temperatures, the pH of whole blood at these two temperatures can be found by finding the plasma pH values of the particular samples at these temperatures. The plasma pH values are plotted against temperature. By drawing a line through each slope, the temperature coefficient of plasma can be drawn. By joining the two points (of temperature of separation on the respective lines) the temperature coefficient of whole blood may be obtained.

What is required in practice is the mean of a number of results. This is most easily determined by finding the average difference in plasma pH between a number of duplicate samples taken at the same time but centrifuged at different temperatures. On the graph of pH against temperature the two parallel lines of slope, the temperature coefficient of plasma, are drawn separated by the average differences. Points on these lines at the temperature of separation are found. The straight line joining these gives the average temperature coefficient of whole blood.

TABLE 3

Determination of Separation Coefficient of Bovine Plasma.

Sample	Sep. at 25° C.	Sep. at 0° C.	Difference
	pH	pH	pH
1	7.510	7.565	.055
2	7.535	7.585	.050
3	7.595	7.670	.075
4	7.620	7.675	.055
5	7.555	7.630	.075
6	7.535	7.600	.065
7	7.560	7.630	.070
8	7.615	7.705	.090
9	7.535	7.585	.050
10	7.400	7.450	.050
11	7.545	7.630	.085
12	7.450	7.530	.080
13	7.550	7.595	.045
14	7.605	7.660	.055
15	7.635	7.720	.085
16	7.620	7.690	.070
17	7.610	7.685	.075
18	7.565	7.635	.070
19	7.635	7.720	.085
20	7.565	7.660	.095
			.070

Separation coefficient = .070

Results

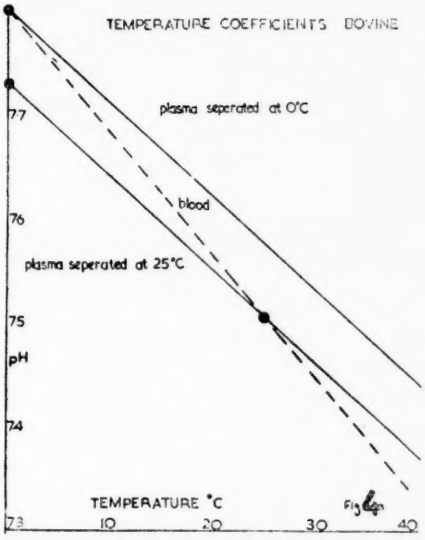
(a) Determination of the true pH at 38°C. of bovine plasma.

Both arterial and venous samples were separated at temperatures of 0°C., 15°C., or 25°C. The colorimeter readings were made at either 20°C. or 38°C. Corrections were made with the previously determined temperature coefficients to obtain the blood pH at 38°C. and thence true plasma pH at this temperature.

E.g. if X be the temperature of separation and Y the temperature of reading pH38°C. = pHY°C. + (X — Y) (.009) — (38 — X) (.012).

(i) True arterial pH (140 samples in duplicate) = 7.43 ± .045 pH units.
(ii) True venous pH (55 samples in duplicate) = 7.39 ± .05 pH units.
Therefore A — V difference = .04 pH units.
(iii) Arterial and venous samples taken at the same time (12 samples).
Arterial pH = 7.45 ± .05 pH units.
Venous pH = 7.41 ± .06 pH units.
Therefore A — V difference = .04 pH units.

FIG 4



(b) Temperature coefficient bovine plasma = 0.009 pH units per degree centigrade. Temperature coefficient bovine blood = 0.012 pH units per degree centigrade.

Therefore temperature coefficient bovine blood = 0.012 pH units per degree centigrade.

(c) For the purpose of comparison temperature coefficients of canine blood and plasma were determined. Identical methods were used. The temperature coefficient of dog blood was found to be .0155 per degree centigrade, that of plasma .012 pH units per degree centigrade.

TABLE 4

Temperature Coefficient of Canine Plasma (10 *samples*).

20° C.		38° C.		
Sample	pH	Sample	pH	Difference
1	7.66	1	7.43	.23
2	7.58	2	7.38	.20
3	7.59	3	7.38	.21
4	7.61	4	7.40	.21
5	7.67	5	7.45	.21
6	7.74	6	7.50	.24
7	7.63	7	7.42	.21
8	7.72	8	7.48	.24
9	7.70	9	7.47	.23
10	7.73	10	7.49	.24

Mean difference = .222 for 18° C.
Therefore coefficient = .012 pH units per degree centigrade.

Using these temperature coefficients the plasma of canine arterial blood was determined, and corrected to 38°C. This figure, 7.41 pH units, is in agreement with that of other authors (Spector, 1956).

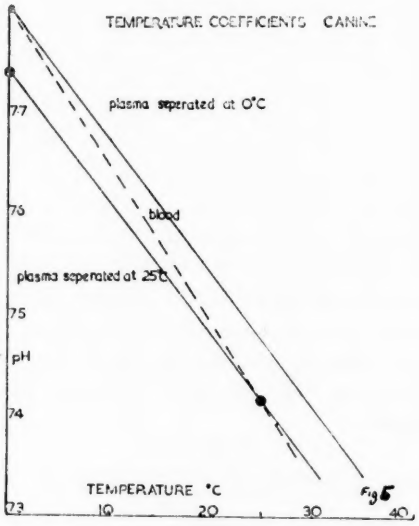
TABLE 5

Temperature Coefficient of Canine Blood (10 *samples*).

Sample	pH 25° C.	Sample	pH 0° C.	Difference
1	7.66	1	7.74	.080
2	7.58	2	7.63	.050
3	7.61	3	7.70	.090
4	7.68	4	7.73	.050
5	7.43	5	7.51	.080
6	7.38	6	7.42	.040
7	7.40	7	7.47	.070
8	7.73	8	7.80	.070
9	7.73	9	7.77	.040
10	7.51	10	7.55	.040

Mean difference due to centrifugation = .061.
Therefore temperature coefficient whole blood = .0155 pH units per degree centigrade.

FIG 5



Discussion

The value obtained for bovine venous plasma pH, 7.39 ± .055 pH units, is in agreement with values published by the majority of other authors. Krapf (1940) using the colorimetric method of Sendroy and Hastings (1924) obtained pH values 7.30 – 7.50.

McSherry and Grinyer (1954) using the method of Sendroy and Hastings (1924) obtained values of pH 7.42 ± .057. Dale and Brody (1954) used a Beckman pH meter

and obtained values of 7.30 – 7.40. Craige *et al.* (1949) used a Beckman pH meter and found values of pH 7.30 – 7.45.

Bianca (1955) used a Marconi pH meter and found values of pH 7.51 and 7.52 in two Ayrshire calves. His values were corrected but were still high. The correction applied was determined for human plasma (Wesson, 1953). The value pH 7.43 ± .043 obtained for arterial plasma is as expected higher than the venous plasma pH.

In the human, although the normal plasma pH is given as 7.4 with a range of pH values 7.35 – 7.45, where arterial and venous pH values are determined together, arterial pH values are usually given in the region of 7.43 – 7.45 with an A – V difference of .04 pH units.

Comparison of the results obtained with those of the dog (pH 7.41 ± .06 pH units) and the human confirm that the bovine, like all other mammals, regulates the pH of its plasma to range about a value of 7.4 pH units.

Summary

(1) Bovine arterial plasma pH at 38°C. has been determined colorimetrically in duplicate on 140 blood samples. A mean value of 7.43 ± .045 units was obtained with a range of 7.33 – 7.54 pH units.

(2) Venous plasma pH at 38°C. has been determined colorimetrically in duplicate on 55 samples. A mean value of 7.39 ± .055 pH units was obtained with a range of 7.30 – 7.51.

(3) Temperature coefficients were obtained for bovine blood and plasma.
Temperature coefficient plasma = .009 pH units per degree centigrade.
Temperature coefficient blood = .012 pH units per degree centigrade.

(4) Comparative determinations were made on arterial blood samples from 10 dogs.
Arterial plasma pH = 7.41 ± .06 pH units with a range of 7.33 – 7.48 at 38°C.
Temperature coefficient plasma = .012 pH units per degree centigrade.
Temperature coefficient blood = .0155 pH units per degree centigrade.

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XUM

A DEVICE FOR POSITIONING SMALL ANIMALS FOR SURGERY*

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One of the major problems confronting those who perform surgical operations on animals is the control and accurate positioning of the animal on the operating bed or table. This difficulty arises because animals placed in the dorsal position are inclined to roll over to a lateral position. My subsequent remarks in this note apply to the small animal only.

Various methods to maintain the dorsal position have been devised. The commonest of these is to hobble the limbs and put the animal in a " spread-eagled " position on the table, the distal ends of the hobbles being secured in cleats or on hooks along the side of the table. There is a risk of injury by over-stretching the limbs and the brachial plexus may be damaged. Sand bags can be used in association with this method in order to give greater stability. Another method is to use a special operating table with an adjustable top. The top can be adjusted so as to produce a variable V cross-section as required. This type of table is expensive and there are difficulties with regard to sterilization.

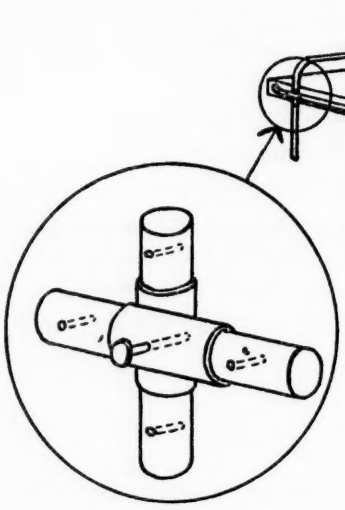

DIAGRAM II
Detail of bar clamps

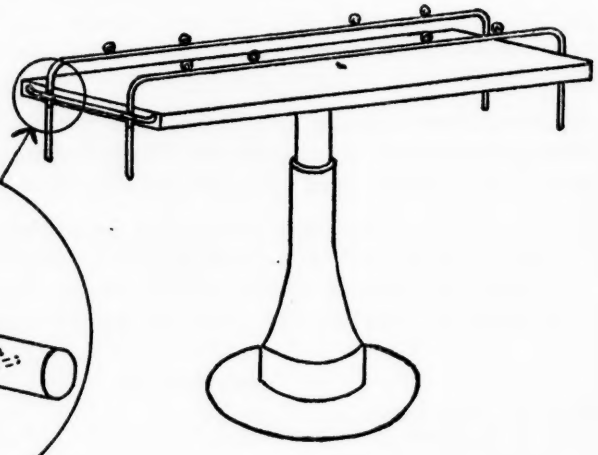

DIAGRAM I
Overall view of operating table fitted with positioning bars

The device described in this note is a simple piece of equipment that with very little trouble can be made to fit most of the small animal operating tables in current use. Basically it consists of two tubular bars which are slightly longer than the length of the table. (Diagram I). A number of eyelets are welded to the top surface of the

* Central Veterinary Society on 6th March, 1958.

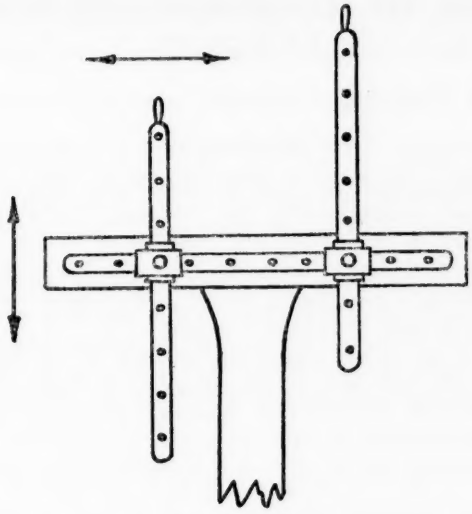

DIAGRAM III

End view of table showing fixing of positioning bars and direction of movements

horizontal part in order to secure the hobbles and limbs. The vertical sections have a series of regular holes drilled through the diameter of the tube. A horizontal bar is fastened at each end of the table. The bar is drilled with holes (Diagram III). The horizontal bar and the ends of the two longitudinal bars are connected by simple clamps (Diagram II). These are composed of two short sleeves welded together at right angles to each other and drilled in a similar manner in order to take a locking pin.

By moving the bars and locking pins a range of positions can be obtained to accommodate animals of all sizes between the bars.

Apart from the pure positioning of the animal on the table there are other uses. The limbs may be fastened directly to the bars in various orthopaedic procedures including the reduction of dislocations and fractures. When transfusion therapy is required the bottle stand can be clamped directly on to the bar and when intermittent intravenous injections are being given both leg and syringe can be taped to a bar. By the use of canvas slings animals can be lifted off the table surface either in the dorsal or ventral position and the sling and the bars can be used as a stretcher to transfer the animal to a recovery ward.

In my opinion this equipment has many advantages and is worthy of further development. With some modifications this device could be adapted to make an easily transportable surgical unit. It is possible that it could be adapted for use with pigs and sheep.

Addendum: Since presenting this item to the Central Veterinary Society I have been interested to read that Henry Gray had devised what would appear to be a similar piece of equipment which he described as a "portable operating rack" (McCunn, 1953).

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XUM

CONTROL OF HAEMORRHAGE IN CANINE SURGERY

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Introduction

Even in ordinary surgical cases there is a need for an agent which is effective in controlling venous and capillary bleeding, thus producing a clearer operating field, providing its administration can be included conveniently in the usual preoperative technique. Such an agent would however be of even more value in those cases where haemorrhage is already a complicating factor, such causes as internal or external injuries, parturition accidents or warfarin poisoning.

Although there has been a number of references in the American veterinary literature (Blamey, 1951; Klausman, 1951; Klausman & Brown, 1952; Rachman & Frucht, 1954; Scott, 1953; Sternfels, 1955; Whitney, 1953) on the value of a parenteral coagulant containing solutions of oxalic and malonic acids, (Archer, 1957) concluded that this type of coagulant had no effect in reducing the clotting time of the blood of the normal horse.

In view of this apparent conflicting evidence, it was decided to investigate the action of a similar parenteral coagulant (Noracin) in canine surgery, to ascertain its effect on the clotting time of the blood of dogs, and to see if haemorrhage was in fact reduced. The mode of action of minimal oxalate and malonate dosage in reducing the clotting time of blood does not appear to be understood but it is postulated that the normal physiological response to the hypocalcaemia induced by the injected oxalate and malonate ions would be expected to be a rapid mobilisation of the calcium reserves. The following notes have been compiled as a result of 14 cases treated with this coagulant.

Method of Use

Preoperative techniques were carried out in the usual way and the operation sites and the sites of intravenous injections were prepared. When everything was ready, the patient was given the normal dose of the anaesthetic. When the animal was anaesthetised the tourniquet was reapplied to the leg and a sample of blood was taken from the intravenous needle, directly by capilliary attraction, into a Dale & Laidlaw tube.

Whilst this was being done an assistant prepared a basin of water at 101.5°F. and as soon as the sample was obtained, the Dale & Laidlow tube was dipped periodically into the water for a few seconds, and examined to see if the shot in the tube was still free to move. This was continued until the shot stopped rolling within the tube; the time taken from the first dipping until immobility occurred being the time required for the blood to coagulate.

At this stage 2 ml. of the parenteral coagulant were injected deep into the muscle at a prepared site on the hind leg and the operation then commenced. The time of injection was noted and a further sample of blood was taken in the manner described previously after 30 minutes had elapsed. The sample was treated in the same way as the previous one and the time taken to coagulate was noted.

XUM

Findings

Coagulation times, breed, type of condition etc. were noted and compiled in tabular form.

Operation Area

From actual experience it appeared that the site of operation was less masked with blood, and the necessity for continuous swabbing was reduced, in cases when the parenteral coagulant had been used. This was a distinct advantage. At this stage it is worth noting that in several cases, when the parenteral coagulant was NOT used preoperatively, profuse bleeding occurred postoperatively, the injection of 2 cc Noracin intramuscularly reduced the haemorrhage considerably. This occurred (i) in two cases of mammary tumours in which there was haemorrhage from minor blood vessels which were too small to be ligatured, (ii) in one case of haemorrhage in a bitch which had just been spayed and was violent while recovering, and (iii) in a case of a vaginal polypus from which there was profuse haemorrhage prior to its discovery and diagnosis.

Breed

So far as can be ascertained from the number of tests carried out it would appear that variation in coagulation time was not associated with breed (Fig. 1).

Sex

It would appear that the difference in coagulation time was about as variable in one sex as in the other (Fig. 2).

Age

No variation of a constant nature was associated with the age of the animal, e.g., a 9 year old's blood coagulated in one case in 63 seconds while only 10 seconds were required in the case of an 11 year old dog and, in a youngster of only 1 year, the variation was only 23 seconds.

Medical Condition

No notable variation was noted between the normal healthy dogs and the seriously ill animals. For example, in a Bulldog Bitch with a tumified harderian gland the blood coagulated 32 seconds under normal time and that of an advanced pyometra case was reduced by 29 seconds. On the whole the variations were so slight that one could not say with the evidence of this limited investigation that the clinical condition notably affected the coagulation time.

Anaesthetic Used

As wide a range as possible was covered but, here again, Fig. 1, shows no appreciable alteration with the anaesthetic used.

Typical Case Histories

Case No. 1. Breed: Cairn Terrier. Sex: Bitch. Condition: Cystic Calculus. History: The bitch had been straining for some weeks while passing urine until the time was reached when she was passing what appeared to be pus admixed with blood. At this stage professional advice was sought. Examination: Respiration was normal. Temperature 103°F. Heart: Physically normal but beating faster than under ordinary conditions. Pulse: 120.

In view of the fact that the animal was rapidly becoming toxaemic and uraemic, immediate operation was advised.

Anaesthetic: The animal was anaesthetised with Themalon intravenously and a state of deep narcosis produced.

Blood sample: At the same time as the Themalon was introduced, a sample of blood was taken direct from the vein into a Dale & Laidlaw tube. This was dipped intermittently into water at 101.5°F. until it coagulated and the time taken was noted. The time taken to coagulate was 45 seconds. Noracin (2 ml.) was then given intramuscularly.

Then the operation was commenced. It took about 25 minutes to complete. At 30 minutes from the time of giving the Noracin a further sample was taken from the vein and treated as the previous one. On this occasion the coagulation time was 15 seconds so that it had been reduced by 30 seconds. The operation was successful and the animal recovered completely.

FIG. 1 *Breed*

Breed	Normal Coagulation	Coagulation after Noracin	Decrease	Average Reduction
Terrier Types	45	15	30	
	46	17	29	
	43	20	23	26
	40	30	10	
	60	30	30	
	45	30	15	
	90	44	46	
Spaniel	75	35	40	
	54	30	24	32
Bulldog (1 case only)	58	26	32	32
Alsatian (1 case only)	63	28	35	35

FIG. 2 *Sex*

Bitch	45	15	30	
	46	17	29	
	43	20	23	
	40	30	10	32
	90	27	63	
	90	44	46	
	49	20	29	
	58	26	32	
Dog	60	30	30	
	75	35	40	
	50	27	23	28
	45	30	15	
	63	28	35	

Case No. 3. Breed: X Terrier. Sex: Bitch. Age: 9 years. Condition: Pyometra. History: Small salacious discharge slightly blood stained from vagina. Bitch otherwise apparently healthy. No inappetence, vomiting or excessive thirst; temperature was raised slightly 102.5°F. Examination: Heart, respiration, pulse were normal.

Abdomen was slightly enlarged and doughy on palpation. Examination per rectum indicated that the neck of uterus was slightly enlarged. Surgical intervention was advised. Anaesthetic: The animal was anaesthetised by a normal intravenous dose of Pentolissin. Blood sample: At the same time a sample of blood was taken in a Dale & Laidlaw tube and treated in water at 101.5°F. until the blood coagulated. This took 43 seconds after which 2 cc Noracin were administered intramuscularly.

The operation then commenced and 30 minutes later a further blood sample was taken from the vein and treated as before. In this case coagulation occurred after treatment for 20 seconds so reducing the time by 23 seconds. The operation was concluded successfully and the animal recovered.

Conclusions

It can be said that 14 cases are insufficient to assess completely the value of any product but the following conclusions have been reached, subject to more cases proving anything to the contrary:—

1. Sex, age, breed, anaesthetics and condition either bodily or medically, do not appear to affect in the least the efficiency of the coagulant in vivo;
2. From experience gained in surgical work both with and without the parenteral coagulant, much advantage is to be gained by its use, not only because of the reduction of haemorrhage in the operation field, and the reduction and control of haemorrhage occasioned in accidental injury, but there is also a use for the preparation in haemorrhage from causes other than those of a surgical nature;
3. It would appear that the coagulation time for blood depends on the constituents of the blood within the individual animal and that nothing else has any influence on the action of the parenteral coagulant.

Acknowledgments

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XUM

THE USE OF THE ZIMMERMANN REACTION FOR ANDROGENS AND 17-KETOSTEROIDS AS A POSSIBLE GUIDE TO SEXING CHICKS

By

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Introduction

A method for sexing chicks was discovered by two Japanese scientists, Kiyoshi Masui and Juro Hashimoto of the Tokyo Imperial University. It depends mainly upon the appearance of the wrinkles of the mucous membrane of the cloaca. The best time to determine the sex is twelve hours after hatching or as soon as they have dried off and have become strong enough to be handled. After chicks are one day old, they are not so easy to sex. The technique calls for much skill and practice. It cannot be carried out after the chicks are 24 hours old and the diagnosis is not correct always.

The early identification of the sexes makes it possible for the poultrymen to place as large a portion of the cockerels as he desires on a special fattening ration and dispose of them as broilers much earlier than would otherwise be possible and reduce the cost of maintenance. Forced feeding is not good for pullets and cockerels which are reserved for breeding purposes. These birds can be kept to better advantage if they are separated from the broiler cockerels.

Pullets grow more uniformly if they are parted from the cockerels at the time of hatching and are kept separate until, as mature birds, they are placed in the breeding pens. Losses from all causes can be reduced in the pullets, if the sexes are reared separately.

It has been established that androgens are produced mainly in two tissues. Well-defined pure substances possessing adrogenic activity have been isolated and identified from the testicular and adrenal tissue. Androgenic substances are elaborated in ovarian and placental tissue also.

Androgens have been studied in the urine of normal individuals and in the urine of those suffering from various pathological conditions. Human urines contain relatively high concentrations of androgens. These substances have been demonstrated in the urine of men, women and children of both sexes. In addition a number of sterioids have been isolated which possess no biological activity, yet are chemically closely related to the androgens. In some cases these biologically inert substances have been shown to be derived from androgenically active material.

Metabolic products of testosterone have a ketone group in the C_{17} position of the steroid nucleus and the 17-ketosteroid concentration in the urine is a useful index of the endogenous production of androgenic hormones. The testes of some animals are capable of producing some C_{21} steroids which can be degraded to 17-ketosteroids.

Determination of steroid substances in urine involves hydrolysis of their glucuronides or sulphates, followed by extraction of the free steroids, fractionation of the extract, and, finally estimation by biological, chemical or physical methods. The separation of estrogens from androgens by utilizing the phenolic character of the former, the development of Girard's reagent for separating ketonic and non-ketonic substances, and digitonin precipitation are among the many noteworthy contributions which made possible a more definite system of fractionation. For the final estimation, the Zimmermann reaction is commonly used. As Holtorff and Koch, Callow and Emmens, and Marlow recognized, colorimetric determinations of neutral 17-Ketosteroids yield higher values than biological assays for androgens, and they give more complete information concerning excretion of steroids which differ both quantitatively and qualitatively in biological activity.

The Zimmermann reaction has been modified by different workers by varying the concentrations of alcohol, m-dinitrobenzone and potassium hydroxide employed, as well as the duration of colour development. The principal techniques have been reviewed and evaluated by Mathanson and Wilson.

The awakening of sex interest and the rapid acceleration of sex activity in animals during the puberal period are correlated with the increased androgen secretion by the testes at this time, and in part are due most likely to increasing levels of this hormone. The author suggests that the application of the above fact may be made use of in order to differentiate between the male and female chicks.

Experimental and Results

Twenty Fayoumi chicks were used.

Technique—A complete 48-hour specimen of faeces and urine of 3-days old chick is collected. No preservative need be added if the specimen is kept refrigerated and is analysed within 24 hours. After recording the total weight of the specimen, it is placed in an Erlenmeyer flask, with a capacity of about 500 ml., diluted with an equal volume of distilled water, treated with 15% by volume of concentrated hydrochloric acid and heated to boiling on a hot plate. When the specimen begins to boil the heating is reduced. Boiling is continued for exactly ten minutes. At the end of this time, the hydrolyzed specimen is cooled rapidly to room temperature under a stream of running water and then transferred to a separating funnel of suitable size. The hydrolyzate is extracted by shaking with four portions of carbon tetrachloride, each one being 20% of the volume of the hydrolyzed sample. Any emulsions which form during the extraction may be broken by centrifugation. Each extraction requires shaking for at least one minute. The carbon tetrachloride solution is then washed, once with 7.5% volume of N-sodium hydroxide solution and once with 3% volume of water.

The carbon tetrachloride is washed with three 50 ml. portions of normal sodium hydroxide and three times with 25 ml. portions of water. The washed CCl_4 solution is then evaporated to dryness under reduced pressure.

To the dry residue exactly 10 ml. of absolute ethanol is added. The flask is swirled to dissolve the residue. When the residue is completely dissolved, it is transferred into a labelled tube and the exact volume marked with a wax pencil. This solution is the total neutral fraction.

XUM

To a colorimeter tube of suitable size the following reagents are added in the order mentioned: 0.2 ml. of the alcoholic solution of the neutral fraction, 0.2 ml. of 2% m-dinitrobenzene in absolute ethanol, and 0.2 ml. of 5N potassium hydroxide solution (aq.). After the reaction mixture has been mixed by gentle agitation, the tube is immersed in a water-bath at $25 \pm 0.2°C$. and kept in the dark.

One hour and forty minutes after the addition of the potassium hydroxide the tube is removed from the bath and dried. 10 ml. of 70% ethanol are then added to the tube and the contents agitated. Colorimeter readings should be made 3-15 minutes after the addition of the 70% ethanol to avoid error due to fading. Readings should be with the 520 and 420 mμ filters.

According to the results obtained the chicks were segregated into two groups. Those who gave dark purple colour by this reaction were presumed to be male and those which gave a light purple colour female chicks. In order to check the above result the chicks were killed and examined directly after death. The chick is placed on its back and the skin is grasped just below the breast bone and torn open exposing the internal organs. By moving the liver the sex organs are exposed.

TABLE I

	+	—	Totals
Female	0	10	10
Male	8	2	10
	8	12	20

It was found that 80% of the male chicks gave a darker purple colour than the females did.

Trials to determine quantitatively the amount of the 17-ketosteroids excreted in 24 hours by male and female chicks are now in progress. Different kinds of chicks will be used in a large scale experiment during the winter of 1957-1958 in order to determine the most suitable age for sexing and the effect of the external conditions on the results of the experiment.

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REVIEWS

PALLASKE-SCHMIDEL, PATHOLOGISCH-HISTOLOGISCHE TECHNIK. 1959. 233 Seiten mit 20 Abb. im Text und 16 Mikro-fotos auf 2 Farbtafeln. Ganzleinen DM 39.60.

This is a concise, detailed and eminently practical account, by the Director of the Veterinary Pathological Institute of the Justus Liebig University of Giessen, and by the technical assistant of the corresponding Institute of the Karl Marx University of Leipzig, of the important staining methods used in histopathology. After an illustrated list of instruments and glassware and preparation of solutions, methods of fixation, decalcification, embedding and sectioning are described with great clarity. Following a useful list of fixation and staining methods applicable to the demonstration of various normal and pathological tissue components, the bulk of the book is devoted to a detailed and orderly description of staining methods, special comments being made on particular points of interest or of difficulty. Two plates show 16 colour pictures of typical staining results. Fairly extensive details of staining methods for the nervous system are given, and the methods used for the demonstration of organisms and of inclusion bodies in sections is described. A final section describes the use of the microscope, including phase contrast, dark-ground, fluorescent and polarization methods. There is a list of formulae, and an index.

In the field of histopathological technique, experience counts, and clearly this book is the result of much careful work, as of wide reading. The excellent production of the book is what would be expected of a Paul Parey publication, and only two misprints have been noticed — phäochomocytom in the table of contents, and Biopsie on p. 123. The book would be of particular use to the laboratory technician, but it could be consulted with profit even by the experienced veterinary histopathologist.

EVOLUTION OF THE VETERINARY ART. Professor J. F. Smithcors, D.V.M., Ph.D. London. Bailliere, Tindall & Cox. Price 55/-.

It is reported that Henry Ford said "History is Bunk" and yet he was a great man and a benefactor of mankind. There are others who survey the past with a jaundiced and critical eye and by comparison with modern attainment disparage the achievements of their forebears. In the light of the knowledge available at the present time many of the ideas and conceptions of ancient times appear to have been founded in error, practised without reason and in some cases they can be described only as ludicrous. These are hard judgments and one is led to wonder if a modern critic, bereft of his store of scientific knowledge, could have done any better under the circumstances prevailing in those times. All things are relative to time, place and circumstance and in passing judgment on the past one should bear in mind that what one can do, or how one does a job, depends to a great extent upon the amount and quality of the knowledge or information and skill which are available at the time. Judged by these standards the achievements of the ancients stand in very good measure and, in many ways, they are of relatively better standard than those of their successors. Records show that there have been "doctors" of men and animals since the dawn of history and until quite recent times they attended to the needs of man and animals with equal facility.

The veterinary art is of very ancient origin and the written word in the Egyptian Papyrus of Kahun demonstrates that it was practised almost two thousand years B.C.

Professor Smithcors reviews the development of the veterinary art from prehistoric to modern times. He tells of the state of affairs in ancient India, Arabia, China, Persia, Greece and Rome. He describes the decline of knowledge from high estate to a welter of ignorance and superstition in medieval times, how the torch was

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lighted again in the 15th Century and spluttered and flickered under the burden of Galen, Hippocrates and the ecclesiastics until the latter half of the 19th century when disease was absolved of the accusation of being the consequence of divine wrath, and the theory of spontaneous origin was discounted.

All these things make interesting and instructive reading. A knowledge of the past is essential when we plan for the future and it is good to know that in Professor Smithcors the profession has found a man to carry the mantle of Sir Frederick Smith who did so much to make veterinary history and ancient lore available to all.

There are many excellent illustrations and the Bibliography at the end will light the road for the many whose appetite is " whetted " but not sated.

NEWS

Mr. G. N. Henderson, B.Sc., M.R.C.V.S., formerly veterinary adviser to The Distillers Company (Biochemicals) Limited, has now been appointed manager of the Company's Veterinary Department. Later this year he will represent DC(B)L at the International Veterinary Congress in Madrid.

NOTICES

Parke, Davis & Company, Limited, announce the following revised prices which became effective on Monday, March 9th, 1959.

Chloromycetin Tincture, Aerosol (Veterinary)

Tins of 4 fl. oz.	per tin	42/6	E

Chloromycetin Tincture, Aerosol (Colourless) (Veterinary)

Tins of 3 fl. oz.	per tin	32/6	E

Riddell Products Ltd. announce the introduction of a new antiseptic, Gomaxide Liquid Germicide, which, whilst being extremely efficient in action, has the merit of being very economical in use. It is non toxic, non irritant and it does not stain. It can be used to sterilise instruments and it does not corrode metals.

It is pleasant to use and it has considerable deodourant properties because it contains Chlorophyll. Gomaxide has been incorporated in an antiseptic cream which has given good results in the treatment of wound and infected skin lesions.

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